Materials List for:

Cryosectioning of Contiguous Regions of a Single Mouse Skeletal Muscle for Gene Expression and Histological Analyses

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Materials

Name	Company	Catalog Number	Comments
Cork	VWR Scientific	23420-708	Cut into small squares with a sharp blade.
Plastic coverslip	Fisher Scientific	12-547	Used to orient the muscle during freezing.
Low temperature thermometer	VWR Scientific	89370-158	
2-methylbutane	Sigma	M32631-4L	Caution: hazardous chemical. Store in flammable cabinet.
Embedding resin: "cryomatrix"	Thermo Fisher Scientific	6769006	Other embedding resins can be substituted for cryomatrix.
Cryostat	Thermo Fisher Scientific	microm HM550 with disposable blade carrier	Any working cryostat should be sufficient for the protocol.
Disposable cryostat blade	Thermo Fisher Scientific	3052835	Use an appropriate blade or knife for the cryostat to be used.
RNAse decontamination solution: "RNase Zap"	Thermo Fisher Scientific	AM9780	
Analytical balance	Mettler Toledo	XS64	
Paint brush	Daler Rowney	214900920	Use to handle cryosections. Can be found with in stores with simple art supplies.
Razor blade	VWR Scientific	55411-050	
Microscope slide	VWR Scientific	48311600	
RNA organic extraction reagent: TRIzol	Thermo Fisher Scientific	15596026	Caution: TRIzol is a hazardous chemical. Note: Only organic extraction reagents are recommended for RNA extraction from skeletal muscle.
18 gauge needle	VWR Scientific	BD305185	
22 gauge needle	VWR Scientific	BD305155	
26 gauge needle	VWR Scientific	BD305115	Optional. Can be used for a third round of sample trituration in the RNA extraction protocol.
1 ml syringe	VWR Scientific	BD309659	For very high value samples, a Luer-Lok syringe is recommended (*e.g.*, VWR BD309628).
1-bromo-3-chloropentane (BCP)	Sigma	B9673	
For 70% ethanol in DEPC water: 200 proof alcohol	Decon Laboratories, Inc.	+M18027161M	Mix 35 ml 200 proof alcohol + 15 ml DEPC water.
For 70% ethanol in DEPC water: DEPC-treated water	Thermo Fisher Scientific	AM9922	Mix 35 ml 200 proof alcohol + 15 ml DEPC water.
RNA purification kit: PureLink RNA minikit	Thermo Fisher Scientific	12183018A	Final steps of RNA preparation.

DNase/Rnase-free water	Gibco	10977	DEPC-treated water can also be used.
Spectrophotometer: Nanodrop 2000	Thermo Fisher Scientific	NanoDrop 2000	
Dnase I	Thermo Fisher Scientific	AM2222	Treat purified RNA to remove any DNA contamination before downstream appications.
Hydrophobic pen	Thermo Fisher Scientific	8899	
Dulbecco's PBS	Gibco	14190	PBS for immunofluorescence protocol.
Donkey serum	Jackson ImmunoResearch Laoratories, Inc	017-000-121	Rehydrate normal donkey serum stock according to the manufacturer's instructions, then dilute an aliquot to 5% for immunofluorescence. Normal goat serum can also be used.
eMHC antibody	University of Iowa Developmental Studies Hybridoma Bank	F1.652	
Collagen VI antibody	Fitzgerald Industries	#70R-CR009x	
Donkey anti-rabbit AlexaFluor488	Thermo Fisher Scientific	A21206	
Goat anti-mouse IgG1 AlexaFluor546	Thermo Fisher Scientific	A21123	
DAPI (4',6-diamidino-2-phenylindole)	Thermo Fisher Scientific	D1306	
Aqueous mounting media: Permafluor	Thermo Fisher Scientific	TA-030-FM	Only use mounting media designed for fluorescent applications with anti-fade properties.
Glass coverslip	VWR Scientific	16004-314	Use for mounting slides at the end of immunofluorescence protocl
Image analysis software: ImageProExpress	Media Cybernetics, Inc.	Image-Pro Express, or more advanced products	Freeware ImageJ should also work for manual counting. More advanced software with segmentation abiities may allow partial automation of the process; *e.g.*, ImageProPremier.
Merge and map section images: Photoshop	Adobe	Photoshop	
Cardiotoxin	Sigma	C9659	Sigma C9659 has been discontinued. Other options for cardiotoxin are EMD Millipore #217503; American Custom Chemicals Corp. # BIO0000618; or Ge Script # RP17303; but these have not been validated.
reverse transcription kit: Superscript III First-strand synthesis system	Thermo Fisher Scientific	18080051	Any validated, high quality reverse transcription reagents can be used.
Standard PCR: GoTaq Flexi polymerase system	Promega	M8298	Any validated, high quality Taq polymerase system can be used. If DNA sequencing is to be used for any application downstream of the PCR, then a high fidelity PCR system should be used instead.
SYBR green	Thermo Fisher Scientific	S7585	For use in qPCR when not using a dedicated qPCR master mix. Use with SuperROX (for Applied Biosystems instruments) and GoTaq Flexi polymerase and buffers.

ROX: SuperROX, 15 mM	BioResearch Technologies, Inc. Novato CA	SR-1000-10	SuperROX is more stable in the PCR reaction, so it is preferred for use as a qPCR passive reference dye over ROX (carboxy-X-rhodamine). For qPCR with Applied Biosystems instruments
Real-time PCR	Applied Biosystems	7900HT	